Rapid Estimation of Rubber in Guayule Latex Dispersions

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SPENCE (4) has shown that the rubber of guayule (*Parthenium argentatum* Gray) is recoverable as a latex which yields a product of high purity and superior quality. His observations on the quality of the latex rubber have been confirmed recently by Clark and Place (1). The occurrence of the latex in small separate cells rather than in a continuous duct system makes necessary a fine comminution of the shrub, and as a result the dilute dispersions initially obtained are highly contaminated with nonrubber plant and soil fragments. A microscopic method for the rapid estimation of rubber in these dispersions has been developed.

The microscopic method, which consists essentially in the identification and counting of rubber latex particles in a small volume of dispersion, is advantageous in several respects. It requires a minimum amount of equipment and is rapid, taking from 10 to 20 minutes for a determination. A rapid method is important because of the instability of some dispersions and the need of knowing the approximate concentration of dispersed rubber during a recovery experiment. Furthermore, only a small quantity of dispersion, as little as one drop, is required for the microscopic method, and it is possible to distinguish between dispersed and partly agglomerated latex in the samples. In contrast, the chemical methods of analysis (5, 7) are time-consuming, do not distinguish between dispersed and partly agglomerated latex, and require larger samples and more extensive equipment.

STANDARDIZATION AND PROCEDURE

The method is standardized by making counts on a number of guayule latex dispersions of known rubber content, as determined by duplicate analyses by the tetrabromide method (7). In this method, the dispersions are extracted in a Waring Blendor with benzene, and rubber hydrocarbon is then determined gravimetrically by precipitation as the tetrabromide. Only dispersions free from agglomerated or partly agglomerated rubber particles should be used for the standardization. Since some dispersions are unstable, the microscopic counts should be made at the time the chemical analyses are begun.

A small sample (usually 1 ml.) of a dispersion of known rubber content is mixed with neutral distilled water to give a known dilution in which the rubber latex particles can be accurately counted in a Petroff-Hausser bacteria counter. Probably a hemacytometer would serve as well, although such a chamber was not tested in the present case. Owing to the tendency of the latex particles to accumulate at the surface on standing, the dispersion must be thoroughly agitated, not only just before sampling for dilution but also after dilution, immediately before mounting in the counter. A dilution which gives 2 to 5 recognizable latex particles per square (1/20,000 cu. mm.) has been found most satisfactory. To ensure a uniform film of dispersion for the mount, the usual precautions must be taken, such as carefully adjusting the cover glass and blotting the excess liquid.

For one determination, all identifiable rubber latex particles, irrespective of size (usually 0.4 to 3.5μ in diameter), in 36 squares in each of two mounts are counted. If agreement between the number of particles counted in the two mounts is not within 5%, counting should be continued on a third mount, or on an additional number of mounts until such agreement is obtained. The count is readily made with a magnification of approximately 800 to 900 diameters, such as is obtained with a 4-mm. objective and 20X ocular. Each particle must be brought into clear focus for identification; those too small for identification should not be counted. Since all flow of particles must be eliminated during counting, it is sometimes necessary to readjust the cover glass

Table I. Standardization of Microscopic Method for Estimation of Rubber in Guayule Crude Latex Dispersions

(*N*, average number of latex particles per square; *D*, dilution factor; *C*, concentration of rubber in original dispersion as determined by chemical method; $k = C_s/ND$; *C*, concentration of rubber in original dispersion calculated from relation $C = 1.85\,ND$.)

| | Original Plants | | Extracted Latex Dispersions | | | | | |
De-scrip-tion[a]	Resin[b], %	Rub-ber[b], %	N	D	k	Rubber Micro-scopic, C	Chem-ical, C	Dif-ference, %
						Mg./100 ml.		
YW	...	3.3	3.00	10	1.77	56	53	+5.7
YX	5.9	8.2	2.42	25	1.87	112	113	−0.9
YX	...	7.3	2.87	25	1.87	133	134	−0.7
YX	6.0	8.3	3.67	25	1.84	170	169	+0.6
YX	5.9	8.2	4.08	25	1.93	189	197	−4.1
YX	...	7.3	2.75	50	1.79	254	246	+3.3
YW	6.8	3.6	2.75	50	1.99	254	273	−7.0
YW	6.8	3.6	3.42	50	1.93	316	330	−4.2
YX	5.4	5.2	2.08	100	1.74	385	361	+6.7
MW	9.0	15.0	2.17	100	1.94	401	421	−4.8
YX	5.6	7.7	2.79	100	1.87	516	521	−1.0
YX	5.4	8.7	3.50	100	1.81	648	632	+2.5
YX	6.8	8.6	3.54	100	1.96	655	694	−5.6
YX	...	8.0	3.92	100	1.82	725	715	+1.4
YX	...	8.0	4.25	100	1.83	786	779	+0.9
MW	9.0	15.0	4.83	100	1.89	894	911	−1.9
YX	5.4	8.7	2.67	200	1.78	988	947	+4.3
YX	...	8.0	2.58	1000	1.74	4773	4501	+6.0
				Av.	1.85			±3.4

[a] Y, 2-year old plants; M, 12-year old plants. X, defoliated plants; W, whole or nondefoliated plants. Data in first three columns indicate range in applicability of method rather than any relationship between original rubber content and amount of rubber extracted.
[b] Moisture-free basis.

carefully. Often slight pressure on the margin of the cover glass will suffice. The predominantly spherical rubber latex particles can readily be distinguished from the variously shaped and colored extraneous nonrubber matter, such as cell-wall fragments, protoplasmic granules, chloroplasts, soap particles, bacteria, and silt. If difficulty is experienced initially in identifying the rubber particles, a portion of them may be stained on a separate slide with a rubber stain (6). Staining, however, coagulates the particles.

Standardization of the method is illustrated by Table I. The observed average number of particles per square, *N*, in the diluted dispersion, the dilution factor *D*, and the rubber content of the undiluted dispersion, C_s, the latter determined by the chemical method of analysis (7), are shown in columns 4, 5, and 8. Constant *k* in column 6 is the ratio of the concentration of rubber in the diluted dispersion (C_s/D) to the average number of particles per square, or $k = C_s/ND$. The average value of *k* for a number of dispersions, as shown in the table, was 1.85. Thus the standardization of the method for the guayule latex dispersions is expressed by the relation $C = 1.85\,ND$.

The quantity of rubber in an unknown dispersion is determined microscopically by counting the particles in an appropriate dilution of the dispersion in a manner identical with that employed during standardization. The concentration of rubber in the original dispersion, in mg. per 100 ml., is calculated by the equation $C = 1.85\,ND$.

DISCUSSION

The concentration of rubber calculated from the microscopic count (column 7) using the relation $C = 1.85\,ND$ and the concentration determined by the chemical method (column 8) are tabulated in Table I. The average per cent difference between the two methods (±3.4) is sufficiently small to establish the validity of the microscopic method. In practice, in some cases where the results obtained by the two methods differed rather widely, a repeated chemical analysis showed the results of the original chemical analysis to be in error. In other cases, differences in results between the two methods may be due to the fact that the chemical method, unlike the microscopic, does not differentiate between dispersed and agglomerated rubber.

The error produced by failure to count the submicroscopic rubber particles is negligible, since these particles, although numerous, constitute only an insignificant fraction of the total rubber. Studies in this laboratory of electron micrographs of kok-saghyz latex show that only 2% of the total volume of all particles occurs in particles smaller than 0.3μ in diameter, although by number these particles constitute 87% of the total. Similar results were obtained by Lucas (3) on hevea latex. The authors' findings and those of Spence (4) indicate that the rubber latex particles of guayule are larger than those of kok-saghyz and hevea.

It would be possible to standardize the microscopic method independently of the chemical method if the average particle mass and the proportion of rubber hydrocarbon in the particles were known. No complete standardization of this sort was attempted in the present case. As a test of its feasibility, however, a preliminary study of the particle size distribution was made visually by means of a micrometer eyepiece. The average particle mass was 9.9×10^{-13} gram. This figure was calculated after measuring the diameters of 600 particles and assuming that they were spheres with a density of 0.92 (2).

The concentration of rubber in a dispersion is expressed, as before, by $C = kND$, but now the constant *k* is equal to *Apm*, where *m* is the average particle mass, *p* is the proportion of rubber hydrocarbon in the particles, and *A* is a constant 2×10^{12} dependent only on the units and on the volume used for determining *N*. In the present case the value calculated for *k* is 1.98 if *p* is assumed to be unity (100% rubber hydrocarbon), or 1.73 if *p* is assumed to be 0.875, a value postulated by Kemp (2) for hevea particles. These values of *k* are in good agreement with the value 1.85 found by standardization in terms of the chemical method. The results indicate that an accurate standardization could be carried out which would be independent of any chemical method except for a determination of *p*.

The wide applicability of the microscopic method to various types of guayule latex dispersions is illustrated by the data of Table I and by subsequent analysis of many samples, in which frequent comparisons with the chemical method were made. Dispersions examined included those extracted in various aqueous media from 2- and 12-year old plants, from whole and defoliated shrub, from fresh and stored plants, and from plants of both high and low resin and rubber content. Agreement with the chemical method under these varying conditions indicated that the rubber latex particles were readily distinguishable from any other particles present, and further that the size distribution and composition of these rubber particles were remarkably constant. On storage after extraction, however, the particles may change in size and possibly composition.

The microscopic method of analysis has been employed in various types of research on guayule. By means of small-scale experiments in which pieces of living guayule tissue were ground in a mortar in anticoagulant solutions and the resulting latex dispersions were analyzed microscopically, significant information on the distribution of latex in the plant and on the effect of pH, enzymes, soaking, temperature, surface active agents, storage, etc., on latex extractions has been obtained. The microscopic method has been applied also in evaluating the effectiveness of each of a series of steps in large-scale latex recovery experiments. With appropriate standardization and with suitable extraction of latex, the method may be applied as a rapid quantitative test for rubber in selection, breeding, and recovery work on other rubber-bearing plants. The data of Lucas (3), showing that about 90% of the rubber in hevea latex occurs in particles 0.4 μ or larger in diameter, suggest that the method may be useful also in the rapid analysis of hevea latex.

ACKNOWLEDGMENT

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